iBiology.org Teaching Tools
Richard Losick's Lecture Part 3:
Stochasticity and Cell Fate

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1. Review Questions

1. **Define the following:**
    a. **Stochasticity**
    b. **Competence**
    c. **Positive feedback loop**
    d. **Pellicle**
    e. **Extracellular matrix**
2. **Explain the concept of "bet hedging" and how it applies to the first three examples of stochasticity given in this lecture**
3. **Explain the concept of altruism and how it applies to the example of stochasticity in the choice between "Individualism vs. Community"**
4. **True or False: All cell fate decisions are made by deterministic processes.**
5. **True or False: Bistability describes a situation in which two distinct cell states can exist stably in a single population.**

6. **Cells mutant for the cannibalism pathway sporulate more quickly.**

7. **Briefly describe two examples of stochasticity in cell fate determination in eukaryotic organisms?**

2. Answers to Review Questions

1.

a. **Randomness**
b. **A state in which bacteria stop growing and become "competent" to take up DNA from their environment**
c. **A circuit in which a regulator stimulates its own synthesis and/or activity**
d. **A community of bacterial cells with complex architecture that forms at an air-liquid interface**
e. **A secreted "cement" composed of polysaccharides and protein that glues bacterial cells to one another and/or to a solid surface**

2. **Bet hedging describes a strategy in which one bets on more than one possible outcome, thus guarding against loss. Bet hedging may explain why subsets of cells in a single population would pursue different cell fates. In the case of swimming vs. chaining bacteria, for example, it may be advantageous for the population to have two subsets of cells: one that stays put, taking advantage of the local conditions, and a second that explores new territories to find new sources of nutrients. In this way, the population is "hedging its bets" on whether or not local conditions will stay favorable.**

3. **Altruism is defined as the "unselfish concern for the welfare of others". In the "Individualism vs. Community" section of the lecture, it is demonstrated that only a subset of the population produces extracellular matrix that is utilized by all cells present in the biofilm. These extracellular matrix-producing cells can therefore be thought of as altruistic.**

4. **False. While the majority of cell fate decisions are not left to chance, several examples of stochastic cell fate decisions have emerged, including those presented in this lecture.**

5. **True.**

6. **True. The nutrients released by killed sibling cells delay sporulation in the surviving cells. In the absence of cannibalism, therefore, sporulation proceeds more quickly.**

7.
   a. **Mouse olfactory neurons. Each mouse olfactory neuron expresses one and only one olfactory receptor gene (out of 2000 total!). The expressed gene is chosen stochastically.**
   b. **Fly ommatidia. Each ommatidia in a fly eye expresses one and only one of two rhodopsin proteins (green or blue). The choice is stochastic, albeit with a bias toward the green rhodopsin, such that the ratio of green:blue ~ 7:3.**

3. Discussion Questions

1. **Biological noise plays an important role in stochastic cell fate decisions. Define noise and speculate as to the potential sources of noise.**

2. **Although noise is usually involved in stochastic cell fate decisions, it is not sufficient. Why not, and what else is required?**

3. **The gene that encodes the Green Fluorescent Protein (GFP) is often used as a reporter gene given that it can be easily observed by fluorescence microscopy. As seen in this lecture, GFP is a popular reporter gene in studies of stochastic cell fate. Another gene called lacZ, which encodes an enzyme called β-galactosidase, is also a well-known reporter gene. β-galactosidase can be easily quantified in cell extracts by enzyme assays. Why is lacZ NOT an ideal reporter gene for studies of stochasticity in cell fate?**

4. **As discussed in the lecture, a positive feedback loop is one mechanism by which small fluctuations caused by noise can be amplified. A second mechanism seen in nature involves a pair of mutually repressing repressors: Repressor 1 (R1) represses transcription of the gene encoding Repressor 2 (R2) and vice versa. Think through this circuit and explain how it would amplify a small change in the level of R1 or R2 due to noise.**

5. **Explain the concept of cooperativity and how it accounts for the ultrasensitivity of the comK promoter to small increases in ComK protein levels.**

6. **What is the possible advantage of stochasticity in the choice of olfactory receptors in the mouse?**

4. Answers to Discussion Questions

1. **Noise refers to small and random fluctuations in the levels of key biological molecules or regulators. Noise can arise from multiple sources, including: differences in the activity or levels of expression of individual genes (due to differences in transcription and/or translation rates, for example), cell-to-cell variations in metabolism, or fluctuating levels of an extracellular signal.**

2. **Biological noise is not sufficient for stochastic cell fate decisions because the resulting fluctuations are small and transient. As such, an important additional requirement is a mechanism to amplify the biological noise and then stabilize the resulting choice. For example, the sensitized circuit described for ComK activation amplifies small fluctuations in ComK levels (due, perhaps, to small changes in the expression of the comK gene), and then locks the cell into a ComK-ON state. Features of the ComK circuit important for this include a positive feedback loop and cooperativity.**

3. **Answers: lacZ is not an appropriate reporter gene because it reports gene activity on a population-based scale, rather than on a cell-by-cell scale (like gfp). β-galactosidase activity is measured in whole cell lysates and therefore only gives a measure of average gene activity per cell. For example, imagine that a given population of bacteria displays 50 units of β-galactosidase activity per cell. One cannot distinguish between the possibility that (1) all cells in fact express 50 units or (2) that half the population expresses no lacZ (0 units) while the other half expresses 100 units (thus averaging to 50 units). (Of course there are infinite possibilities in addition to these two.) Since stochastic cell fate studies, by definition, involve cell-to-cell differences, they require reporter genes that can be measured at the individual cell level, such as gfp.**

4. **Answers: Imagine a small increase in the level of R1. This would cause increased repression of the gene encoding R2, thus decreasing R2 protein levels. As a result, the gene encoding R1 would be de-repressed (because less R2 repressing it), resulting in even higher levels of R1. This cycle would be self-reinforcing, resulting ultimately in R1-ON and R2-OFF. Because a small increase in R1 results in more R1**

production (albeit indirectly), this is essentially equivalent to a positive feedback loop.

5. **Answers: Cooperativity arises from the interaction of two or more DNA-binding proteins bound to adjacent sites in DNA with each other. The favorable free energy from protein-protein interactions augments the favorable free energy from the binding of the proteins to the DNA, thereby stabilizing the adherence of the proteins to the DNA. Therefore, a small increase in protein levels can have a disproportionate (non-linear) effect of the binding of ComK to DNA. Expression of comK gene is therefore ultrasensitive to small increases in ComK levels, which in turn triggers a self-reinforcing cycle of ComK synthesis.**

6. **Answers: The genome of the mouse has ~2,000 olfactory receptor genes. Yet, discrimination among odorants requires that each neuron express only one receptor gene. A regulatory circuit that was capable of ensuring that all 2000 receptor genes are expressed in different neurons would be highly complex. Stochasticity achieves the same goal but in a simple manner. The choice of receptor is made stochastically in individual neurons such that the activation of any one gene by a random process that prevents the expression of all others. Thus, among the entire population of neurons all receptors will be expressed yet only one receptor is expressed in any one neuron.**

5. Explain or Teach These Concepts to a Friend

1. **Describe the circuitry that allows some B. subtilis cells to be ComK-ON and others to be ComK-OFF. You should use the words "positive feedback loop", "threshold", "noise", and "cooperativity" in your explanation.**
2. **Explain why it may be advantageous for a bacterial population to harbor two or more subpopulations with different cell fates/characteristics. Are there disadvantages to this strategy?**
3. **Explain why the precise levels of expression of the same genes varies from cell to cell in an otherwise homogeneous population of cells. Describe two possible sources of noise in gene expression.**

4. **The fly is said to have a compound eye. Why? Are color receptor genes expressed in a uniform pattern across the fly retina? Explain.**

6. Research the Literature on your Own

1. **What is the difference between extrinsic and intrinsic noise? Design an experiment that distinguishes between the two.**
2. **Define "epigenetic". Are stochastic cell fate decisions epigenetic? Why or why not?**
3. **A classic example of a bistable switch is that governing the choice between lysogeny and lytic growth by the bacterial virus lambda. Describe the circuitry governing these alternative states and explain the role of cooperative interactions among phage lambda repressor proteins in the circuit.**
4. **Bacteria spontaneously enter a slow or non-growing "persister" state in which they are resistant to antibiotics. Investigate how this was demonstrated using microfluidics.**
5. **The pathogen Candida albicans switches between two phenotypic states called white and opaque. Is switching genetic or epigenetic and what is the mechanism of switching?**
6. **In the mouse olfactory system, a single odorant receptor gene is expressed in any given neuron to the exclusion of all of other ~2,000 receptor genes. What is the mechanism?**

7. Papers for Journal Club

These two reviews would serve as a good starting point for delving deeper into the concepts and details of stochasticity and cell fate in bacteria and higher organisms.

- **Dubnau D and Losick R (2006) Bistability in bacteria. Mol Microbiol 61: 564-572.**
- **Losick R and Desplan C (2008) Stochasticity and cell fate. Science 320: 65-68.**

These three primary research papers, all of which are based on work in the Losick lab, describe the role of stochasticity in B. subtilis motility vs. chaining, cannibalism, and biofilms. These are the studies upon which the corresponding sections of the lecture were based, but will provide more detail and depth as to the experimental evidence for these stochastic cell fate decisions.

- **Kearns DB and Losick R (2005) Cell population heterogeneity during growth of Bacillus subtilis. Genes Dev 19: 3083-3094.**
- **González-Pastor JE, Hobbs EC, and Losick R (2003) Cannibalism by sporulating bacteria. Science 301: 510-3.**
- **Chai Y, Chu F, Kolter R, and Losick R (2008) Bistability and biofilm formation in Bacillus subtilis. Mol Microbiol 67: 254-263.**

Development of competence by B. subtilis is arguably one of the best-understood examples of the role of noise, stochasticity, and bistability in cell fate determination. The following papers represent some of the critical work that has formed our current knowledge of this system. In the third and most recent paper, the authors actually observe noise at the level of single mRNA molecules, and based on their results, are able to draw conclusions on the source of noise in the competence system.

- **Smits WK, Eschevins CC, Susanna KA, Bron S, Kuipers OP, and Hamoen LW (2005) Stripping Bacillus: ComK auto-stimulation is responsible for the bistable response in competence development. Mol Microbiol 56: 604-614.**
- **Maamar H and Dubnau D (2005) Bistability in the Bacillus subtilis K-state (competence) system requires a positive feedback loop. Mol Microbiol 56: 615-624.**
- **Maamar H, Raj A, and Dubnau D (2007) Noise in gene expression determines cell fate in Bacillus subtilis. Science 317: 526-529.**

These final 3 papers describe research into examples of stochastic cell fate decisions in other bacteria and in complex metazoans, including the example of green vs. blue rhodopsin expression in the fly eye briefly described in the lecture.

Escherichia coli perisister cells:

- **Balaban NQ, Merrin J, Chait R, Kowalik L, and Liebler S (2004) Bacterial persistence as a phenotypic switch. Science 35: 1622-1625.**

- **Keren I, Shah , Spoering A, Kaldalu N, and Lewis K (2004) Specialized persister cells and the mechanism of multidrug tolerance in Escherichia coli. J Bacteriol 186: 8172-8180.**

Stochasticity and the fly eye:

- **Wernet MF, Mazzoni EO, Celik A, Duncan DM, Duncan I, and Desplan C (2006) Stochastic spineless expression creates the retinal mosaic for colour vision. Nature 440: 174-180.**